STATUS OF CLAIMS:

Claims 1-16, 18-23, 29-31, 33-44, 46, 50, 52 and 53 remain pending.

REMARKS:

As a preliminary matter, the Examiner is thanked for the courtesies extended in the telephonic interview with Michael Moran, Kimberly Denis-Mize and the undersigned on November 4, 2004. During the telephonic interview of November 14, Song et al., Hedley et al. and Fattal et al. were discussed.

Rejection of Claims 1-16, 18-23, 29-31, 33-44, 46, 50, 52 and 53--35 U.S.C. 103(a)

Claims 1-16, 18-23, 29-31, 33-44, 46, 50, 52 and 53 continue to be rejected under 35 U.S.C. 103(a) as obvious over Song et al. in view of Hedley et al. and Fattal et al.

Claim 1, the only independent claim presently pending, reads as follows:

> 1. A method of transfecting dendritic cells comprising:
> providing dendritic cells;
> providing a transfection agent comprising polynucleotide adsorbed on surfaces of microparticles, said transfection agent being formed by a process that comprises: (a) providing microparticles comprising a biodegradable polymer and a cationic detergent, and (b) exposing said microparticles to said polynucleotide, said polynucleotide encoding an antigen associated with a virus, a bacterium, a parasite, a fungus or a tumor; and
> incubating the dendritic cells and the transfection agent *ex vivo* for a time sufficient to transfect the dendritic cells with the polynucleotide, thereby leading to the expression of said antigen.

Claim 1 is unobvious over Song et al. in view of Hedley et al. and Fattal et al.

According to the Office, Song et al. teaches methods of transfecting dendritic cells *ex vivo* or *in vitro* with a gene delivery vehicle comprising DNA encoding an antigen, such as a tumor antigen or HIV antigen, and use of the transfected dendritic cells to induce an immune response against the expressed antigen *in vivo*. Regarding gene delivery vehicles taught by Song et al., the Office agues that Song et al. teaches that for *ex vivo/in vitro* transfection of dendritic cells, both non-viral and viral gene delivery vehicles can be used, including the use of expression vectors complexed with polycations

or lipids or encapsulated in liposomes. The Office concludes that Song et al. teaches that numerous gene delivery vehicles can be successfully utilized to transfect dendritic cells including the use of plasmid/liposomes, and plasmid combined with cationic condensing agents.

The Office acknowledges that Song et al. differs from the present invention by not teaching the claimed combination of polynucleotide, biodegradable polymer and cationic detergent as a transfection agent for dendritic cells. The Office contends, however, that Hedley et al. supplements Song et al. through its teachings regarding the use of microspheres comprising biodegradable polymers and DNA plasmids to introduce and express antigens encoded by the plasmids in antigen presenting cells such as macrophages and dendritic cells, both *in vitro* and *in vivo*, for the purpose of stimulating antigen specific immune responses. It is further argued that Hedley et al. provides motivation for introducing plasmid DNA encoding an antigen to antigen presenting cells such as macrophages and dendritic cells using biodegradable microspheres by teaching that DNA combined with biodegradable microparticles is efficiently phagocytosed by APCs and is an effective means for introducing nucleic acids into these cells. The Office further argues that Hedley et al. recognizes that dendritic cells are a "legitimate target" for microparticle transfection when they state that the point of introduction of plasmid/microparticles to skin is the transfection of dendritic cells.

The Office acknowledges that Song et al. and Hedley et al. differ from the instant invention in that they do not teach the use of microparticles containing cationic detergent to transfect dendritic cells. Fattal is cited by the Office as allegedly providing motivation for including a cationic detergent in a microparticle by teaching that inclusion of a cationic detergent in microparticles increases the amount of polynucleotide associated with the polymer particles and increase the uptake of the nucleic acid by phagocytosis.

These assertions are respectfully traversed for at least the following reasons.

First, the invention of Hedley et al. is said to be based on the discovery that *microparticles* containing nucleic acids and having an appropriate size for phagocytosis can be made without adversely affecting nucleic acid integrity. Col. 1, lines 32-35. At least 99% of the microparticles of Hedley et al. have a diameter less than *100 microns*

(μm). In Example 1 of Hedley et al. approximately 85% of the microparticles were between 1.1 and 10 microns in diameter.

On the other hand, as briefly discussed in the telephonic interview with the Examiner on November 4, 2004, the particles of Fattal et al. are not microparticles like those of Hedley et al., rather they are biodegradable polyalkylcyanoacrylate *nanoparticles*. See Fattal et al. title. The preparation of nanoparticles in Fattal et al. references Courvreur et al (1984), U.S. Patent No. 4,489,055, which describes the methods for making alkyl-cyano-acrylate particles having diameters *less than 500 nanometers (0.5 μm)*. Procedures producing particle sizes of less than 200 nanometers (Example 1), between 300 and 500 nanometers (Example 2), and smaller than 200 nanometers (Example 5) are reported.

It is well known that particle internalization routes are dependent upon the size of the particle. For example, consistent with the teachings of Hedley et al, phagocytosis is defined by others in the art as a "process by which certain cells of the innate immune system, including macrophages and neutrophils, engulf large particles (> 0.5 μm in diameter...)" See Abbas et al., Eds. *Cellular and Molecular Immunology* (4th ed., 2000), attached. See also Mukherjee et al., *Physiological Rev.* Vol. 77, 759-797 (1997), p. 783, attached.

Hence, whereas large particles such as the microparticles of Hedley et al. (i.e., > 0.5 μm) are known to be internalized by phagocytosis, nanoparticles such as those of Fattal et al., being less than 0.5 μm in size, are not.

Because these references are directed to particles which are internalized via different pathways, it is respectfully submitted that one of ordinary skill in the art would not have been motivated to combine the teachings of Hedley et al. with those of Fattal et al., and it is further submitted that any degree of success observed with the nanoparticles of Fattal et al. would not be expected with the microparticles Hedley et al, and vice versa.

These conclusions are buttressed by the fact that Hedley et al. is directed to entrapped nucleic acids, whereas those of Fattal et al. are adsorbed.

Moreover, Fattal et al. reports the internalization of a 15-mer *oligonucleotide* adsorbed onto nanoparticles, and that the oligomer remains intact for several hours after

uptake. Of course, oligonucleotides *per se* do not function in the same manner as nucleic acid expression vectors, such as those described in Hedley et al. which encode and express a polypeptide. It is respectfully reiterated that the mere fact that a 15-mer oligonucleotide *remains intact* upon internalization would not have lead to a reasonable expectation that nucleic acid vectors such as those described in Hedley et al. would be *expressed.*

For at least the above reasons, it is respectfully submitted that one of ordinary skill in the art would not have been motivated to perform the method set forth in claim 1 in view of the teachings of Song et al., Hedley et al. and Fattal et al. and that a *prima facie* case of obviousness has not been established with respect to the presently pending claim 1.

Claims 2-16, 18-23, 29-31, 33-44, 46, 50, 52 and 53 depend from claim 1 and are therefore patentable for at least the same reasons as is claim 1.

Reconsideration and withdrawal of the outstanding rejection under 35 U.S.C. §103(a) are therefore respectfully requested.

CONCLUSION

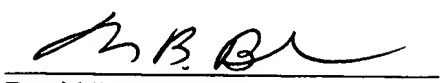
All pending claims are in condition for allowance, notification of which is earnestly solicited. The Examiner is invited to telephone the Applicant's attorney at (703) 433-0510 to resolve any outstanding issues in this case.

CORRESPONDENCE

Please continue to direct all correspondence to:

Chiron Corporation
Intellectual Property-R440
P.O. Box 8097
Emeryville, CA 94662-8097.

Respectfully submitted,



David B. Bonham
Registration No. 34,297

Mayer Fortkort & Williams, PC
251 North Avenue West, 2nd Floor
Westfield, NJ 07090
Tel.: 703-433-0510
Fax: 703-433-2362

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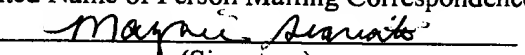

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Endocytosis

SUSHMITA MUKHERJEE, RICHIK N. GHOSH, AND FREDERICK R. MAXFIELD

Department of Biochemistry, Cornell University Medical College, New York, New York

Mukherjee, Sushmita, Richik N. Ghosh, and Frederick R. Maxfield. Endocytosis. *Physiol. Rev.* 77: 759–803, 1997.—Mammalian cells take up extracellular material by a variety of different mechanisms that are collectively termed endocytosis. Endocytic mechanisms serve many important cellular functions including the uptake of extracellular nutrients, regulation of cell-surface receptor expression, maintenance of cell polarity, and antigen presentation. Endocytic pathways are also utilized by viruses, toxins, and symbiotic microorganisms to gain entry into cells. One of the best-characterized endocytic mechanisms is receptor-mediated endocytosis via clathrin-coated pits. This mode of endocytosis constitutes the major emphasis of this review, with a brief discussion of other endocytic mechanisms and their comparison with the receptor-mediated pathway. This review describes and evaluates critically current understanding of the mechanisms of entry of plasma membrane components such as the receptor-ligand complexes and membrane lipids as well as the extracellular fluid into cells. The intracellular sorting and ultimate fate of these molecules upon internalization are also described. The roles of endocytosis in physiological and pathological processes are discussed. These include maintenance of cell polarization, antigen presentation, nutrient transport, atherosclerosis, Alzheimer's disease, and the endocytosis of toxins and viruses.

of GLUT4 expressed at the plasma membrane, which is sustained while insulin is present (462, 463). The GLUT4 molecules continue to recycle in the presence of insulin. An increase in the rate of externalization clearly plays a major role in the altered distribution of GLUT4. However, whether the rate of internalization is also altered is unclear. Unfortunately, it has been difficult to make accurate kinetic measurements to determine the relative contributions of these two processes.

It is currently not known precisely how the recycling itinerary of GLUT4 differs from other recycling molecules such as transferrin receptors. It was recently shown that GLUT4 and VAMP-2 are enriched in a postendocytic compartment distinct from the endocytic recycling compartment (300), but the pathways into and out of this compartment have not been determined.

D. Antigen Presentation

Class II major histocompatibility complex (MHC)-restricted presentation of extracellular antigens under normal conditions is carried out primarily by "antigen presenting cells" that include macrophages, B cells, and dendritic cells (52). During a sustained inflammatory response, however, even fibroblasts and endothelial cells can present class II-restricted antigens, due to the upregulation of class II MHC expression by interferon-γ (52). Foreign (nonself) antigens presented on the cell surface by class II molecules activate helper T cells (53). Intact or native antigen molecules are first degraded or "processed" after internalization into small peptides and are then bound by the MHC molecules for presentation (53). Class II molecules avoid binding peptides during their biosynthesis due to the presence of an additional "invariant" chain that blocks peptide loading in the secretory pathway (52). The invariant chain then targets the class II MHC molecule to intracellular organelles, that are either part of or related to, the endocytic pathway (21, 263, 288). Recent evidence from several groups shows that the compartment for antigen loading, while having many of the characteristics of an endosomal organelle, is physically distinct from the early and the late endosomes and from the dense-core lysosomes (7, 391, 507, 535). A proposed name for this compartment is the "MIIC" (372).

Uptake of an antigen by receptor-mediated endocytosis (e.g., via a surface immunoglobulin) has been reported to cause more efficient T-cell activation than uptake by fluid pinocytosis (265, 375). Furthermore, antigens targeted to the endocytic pathway by different cell surface receptors seem to result in differential efficiency of presentation (375). It has been postulated that although degradation of the antigens to release presentable peptides probably starts in the early endosomal compartments, most of the degradation occurs in the later (more acidic) compartments, giving rise to the complete repertoire of peptides for cell surface presentation (52). In the low pH environment of the endosomes or the MIIC, the invariant chain is released from the class II molecule, thus allowing it to bind the antigenic peptides for presentation (52). The pathway taken by the antigen-loaded class II molecules from these endocytic compartments to the plasma membrane is not clear. Because class II MHC molecules are stable even when they are not bound to any peptides, reinternalization of these "empty" molecules and peptide loading in subsequent internalization steps may also contribute to the overall efficiency of antigen presentation (135, 316).

VI. OTHER (NONCLATHRIN-MEDIATED) MODES OF ENDOCYTOSIS

A. Phagocytosis

Phagocytosis was described by Metchnikoff in the late nineteenth century (318). It is receptor- and actin-dependent and clathrin-independent internalization of large particles and microorganisms (typically >0.5 μm) into a cell (392). A particle that is endocytosed by this mechanism may be recognized directly by the receptors on the phagocyte surface, or it may be first "opsonized" by coating the particle with "opsonins." The major opsonins used by cells are complement components and immunoglobulins (162). Specific receptors recognize the opsonins and engulf the particle. During periods of high phagocytic activity, up to 40% of the plasma membrane may be internalized in 15 min (450). In mammals, phagocytosis is carried out primarily by the so-called "professional phagocytes," which include the neutrophils, monocytes, and macrophages as well as other cells of myelogenic lineage such as microglia in the brain. Although neutrophils, monocytes, and macrophages constitute the main repertoire of phagocytic cells in mammals, other cell types also have varying phagocytic capabilities, many of which are enhanced for cells in culture (118, 151).

The predominant model for ingestion of a particle by phagocytosis is the "zipper model" (163, 164). According to this model, close apposition of a particle to the plasma membrane of a phagocyte is an essential but not sufficient first step for its phagocytosis. Phagocytosis involves the extension of a pseudopod (in the form of a closely fitting sleeve of plasma membrane) around the particle to be engulfed, with a sequential recruitment of cell surface receptors to interact with the native proteins or opsonins on the surface of the particle. As a result, the pseudopod extends only as far as its surface has ligand to bind the receptors, in a way analogous to the teeth of a zipper. Because pseudopod extension is a highly localized event, phagocytosis of one particle does not cause an indiscrimi-

FOURTH EDITION

CELLULAR AND MOLECULAR IMMUNOLOGY

Abul K. Abbas, MBBS

Professor and Chair
Department of Pathology
University of California–San Francisco School of Medicine
San Francisco, California

Andrew H. Lichtman, MD, PhD

Associate Professor of Pathology
Harvard Medical School
Brigham and Women's Hospital
Boston, Massachusetts

Jordan S. Pober, MD, PhD

Professor of Pathology, Immunobiology, and Dermatology
Yale University School of Medicine
New Haven, Connecticut

W.B. SAUNDERS COMPANY

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Philadelphia London New York St. Louis Sydney Toronto

Term	Definition
Periarteriolar lymphoid sheath (PALS)	A cuff of lymphocytes surrounding small arterioles in the spleen, adjacent to lymphoid follicles. A PALS contains mainly T lymphocytes, about two thirds of which are $CD4^+$ and one third are $CD8^+$. In humoral immune responses to protein antigens, B lymphocytes are activated at the interface between the PALS and follicles and then migrate into the follicles to form germinal centers.
Peripheral lymphoid organs/tissues	Organized collections of lymphocytes and accessory cells, including the spleen, lymph nodes, and mucosa-associated lymphoid tissues, where adaptive immune responses are initiated.
Peripheral tolerance	Physiologic unresponsiveness to self antigens that are present in peripheral tissues and not usually in the generative lymphoid organs. Peripheral tolerance is induced by the recognition of antigens without adequate levels of the costimulators required for lymphocyte activation or by persistent and repeated stimulation by these self antigens.
Peyer's patches	Organized lymphoid tissue in the lamina propria of the small intestine where immune responses to ingested antigens may be initiated. Peyer's patches are composed mostly of B cells, with smaller numbers of T cells and accessory cells, all arranged in follicles similar to those found in lymph nodes, often with germinal centers.
Phagocytosis	The process by which certain cells of the innate immune system, including macrophages and neutrophils, engulf large particles (>0.5 μm in diameter) such as intact microbes. The cell surrounds the particle with extensions of its plasma membrane by an energy- and cytoskeleton-dependent process; this process results in the formation of an intracellular vesicle called a phagosome, which contains the ingested particle.
Phagosome	A membrane-bound intracellular vesicle that contains microbes or particulate material from the extracellular environment. Phagosomes are formed during the process of phagocytosis, and fusion with other vesicular structures such as lysosomes leads to enzymatic degradation of the ingested material.
Phosphatase (protein phosphatase)	An enzyme that removes phosphate groups from the side chains of certain amino acid residues of proteins. Protein phosphatases in lymphocytes, such as CD45 or calcineurin, regulate the activity of various signal transduction molecules and transcription factors. Some protein phosphatases may be specific for phosphotyrosine residues and others for phosphoserine and phosphothreonine residues.
Phospholipase C (PLC)	An enzyme that catalyzes hydrolysis of the plasma membrane phospholipid PIP$_2$ to generate two signaling molecules, IP$_3$ and DAG. PLC becomes activated in lymphocytes by antigen binding to the antigen receptor.
Phytohemagglutinin (PHA)	A carbohydrate-binding protein, or lectin, produced by plants that cross-links human T cell surface molecules, including the T cell receptor, thereby inducing polyclonal activation and agglutination of T cells. PHA is frequently used in experimental immunology to study T cell activation. In clinical medicine, PHA is used to assess whether a patient's T cells are functional or to induce T cell mitosis for the purpose of generating karyotypic data.
Plasma cells	A terminally differentiated antibody-secreting B lymphocyte with a characteristic histologic appearance, including an oval shape, eccentric nucleus, and perinuclear halo.
Platelet-activating factor (PAF)	A lipid mediator derived from membrane phospholipids in several cell types, including mast cells and endothelial cells. PAF can cause bronchoconstriction and vascular dilatation and leak and may be an important mediator in asthma.
Pluripotent stem cell	An undifferentiated bone marrow cell that divides continuously and gives rise to additional stem cells and cells of multiple different lineages. A hematopoietic stem cell in the bone marrow will give rise to cells of the lymphoid, myeloid, and erythrocytic lineages.
P-nucleotides	Short inverted repeat nucleotide sequences in the VDJ junctions of rearranged Ig and TCR genes that are generated by hairpin intermediates and contribute to the junctional diversity of antigen receptors.
Polyclonal activators	Agents that are capable of activating many clones of lymphocytes, regardless of their antigen specificities. Examples of polyclonal activators include anti-IgM antibodies for B cells and anti-CD3 antibodies, bacterial superantigens, and PHA for T cells.
Poly-Ig receptor	An Fc receptor expressed by mucosal epithelial cells that mediates the transport of IgA and IgM through the epithelial cells into the intestinal lumen.
Polymerase chain reaction (PCR)	A rapid method of copying and amplifying specific DNA sequences up to about 1 kb in length that is widely used as a preparative and analytical technique in all branches of molecular biology. The method relies on the use of short oligonucleotide primers complementary to the sequences at the ends of the DNA to be amplified and involves repetitive cycles of melting, annealing, and synthesis of DNA.
Polymorphism	The existence of two or more alternative forms, or variants, of a particular gene that are present at stable frequencies in a population. Each common variant of a polymorphic gene is called an allele, and one individual may carry two different alleles of a gene, each inherited from a different parent. The MHC genes are the most polymorphic genes in the mammalian genome.